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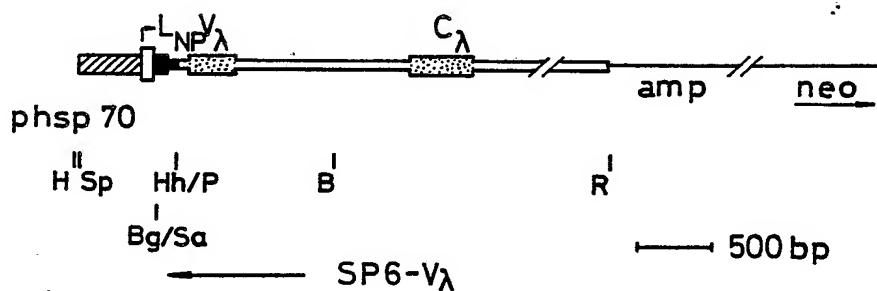
(57) Abstract

There is provided a process for the production of an assembled polymeric recombinant antibody having its constant regions derived from an IgM, characterised in that the process is carried out in the absence of J-chain or any active gene coding therefor. Preferably, the host cell is an actively secreting cell, such as a glial cell, so that the transformed cell can be transplanted into the brain.

Recombinant antibodies and methods for their production.

The present invention relates to the production of IgM
using recombinant DNA technology.

Immunoglobulins (Igs), part of the body's normal defence
system, have been well known for a considerable time.
There are five classes of Ig, being IgA, IgD, IgE, IgG
and IgM. When mounting an immune response to a soluble
antigen, generally signicant amounts of IgM are first
produced. The production subsequently switches towards
production of IgG. Such antibodies can be found
circulating in most body fluids, such as blood and lymph
fluids. However, the antibodies cannot cross the
blood/brain barrier.

IgG is a monomeric species, i.e. it is found as single
units comprising two heavy and two light chains bound
together in conventional fashion by disulphide bonds.
Since IgG is monomeric, it readily attaches to its
antigen. However, it is generally unable to activate
any of the body's effector functions until it is
aggregated.

IgG is used in diagnosis, for instance for blood typing,
but has disadvantages in that it only gives a positive
result if it can be caused to aggregate. It is often
difficult to ensure that the diagnostic test system is
set up to produce aggregation only when a positive
result should be obtained.

IgM is a pentameric species comprising five single
units, similar to IgG units, bound together at the free
ends of the heavy chains. In natural IgM there is a
small protein molecule, known as J chain, associated
with the pentameric unit at the free ends of heavy
chains. There is only one J chain per pentameric unit
and the J chain appears to be located between only two
of the IgM single units. Conventional wisdom among
immunologists is that J chain is essential for enabling
expression, assembly and secretion of pentameric IgM.

Since IgM is a pentameric unit, it is effectively
already aggregated. It therefore finds use in
diagnostic testing and can give a more accurate result
than an IgG based test. However, IgM is relatively less
abundant than IgG. There is therefore a need for a more
abundant supply of IgM.

In recent years, the advent of the monoclonal antibody
technique and the techniques of recombinant DNA
technology has enabled the production of large
quantities of pure Ig. Moreover, it has enabled the
production of "chimeric" antibodies, i.e. antibodies
having a variable region derived from one species and a
constant region derived from another species, "altered"
antibodies, i.e. antibodies having a variable region
from an Ig attached by peptide linkage to a co-expressed
effector protein, and "humanised" antibodies, i.e.
antibodies in which the complementarity determining
regions (CDRs) of an antibody from one species have been
grafted onto the framework regions of the variable
region of an antibody from a different species, usually
a human. All such antibodies are referred to herein
generically as "recombinant" antibodies.

The production of such recombinant antibodies is shown,
for instance, in EP-A-120 694 (Celltech), EP-A-0 125 063
(Genentech), EP-A-0 174 494 (Stanford), EP-A-0 194 276
(Celltech) and EP-A- 0 239 400 (Winter).

Although it has been suggested that such recombinant
antibodies could be expressed in and secreted by any
commonly available prokaryotic or eukaryotic host cell,
such as microbial, yeast or mammalian host cells, it has
generally been found to be necessary to use lymphoid
cells to obtain efficient expression, secretion and
assembly of recombinant antibodies. The most usually
used host cells are myeloma or myeloma-derived host
cells. It has generally been found that recombinant
antibodies are not efficiently expressed, are not
properly assembled or are not secreted when other
commonly available host cells, such as E. coli,
S. cerevisiae, CHO or HeLa cells, are used.

According to a first aspect of the present invention,
there is provided a process for the production of an
assembled polymeric recombinant antibody having its
constant regions derived from an IgM, characterised in
that the process is carried out in the absence of
J-chain or any active gene coding therefor.

The present inventors have surprisingly discovered that
recombinant polymeric IgM-type antibody can be produced
in the absence of J-chain and that such recombinant
polymeric IgM-type antibody can be expressed, assembled
and secreted. This is directly contrary to the
conventional wisdom. It is to be noted that in prior
art procedures for producing recombinant antibodies,
lymphoid cell lines were used as host cells. Such cells
have endogeneous active genes encoding J-chain and

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therefore, although no specific measures may have been taken to produce J-chain, the endogeneous genes produced sufficient J-chain to enable an IgM-type molecule to be assembled in its presence.

The discovery of the present inventors means that it will be possible to express, secrete and assemble a recombinant polymeric IgM-type antibody from any convenient non-lymphoid host cell or lymphoid host cell in which the endogeneous J chain-encoding genes are absent or inactive with the use of only one or at most two expression vectors. There will be no need to produce a vector encoding the J chain. This will clearly simplify the procedure needed to produce a recombinant IgM-type antibody.

Even though it may be possible to use any available host cell for recombinant IgM-type antibody production, it is likely that, in many cases, the amount of production will be low, thus making it difficult to produce the recombinant IgM-type antibody on a commercial scale without the need to resort to the use of lymphoid host cells.

However, the present inventors have discovered that it is possible to use a glioma cell line as a host cell for the production of recombinant polymeric IgM. The glioma cell line is a cancerous cell line derived from glial cells from within the brain. Glial cells are classified as actively secreting cells. It is also possible to use other non-lymphoid actively secreting cells as host cells for the production of recombinant polymeric IgM.

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Among the actively secreting cells which can be used as
host cells are exocrine cells, such as pituitary or
pancreatic cells, and neural cells, such as pheochromal
cells and, in particular, glial cells. Such actively
secreting cells have the advantage that normal
(non-cancerous) cells can readily be cultured in vitro.
They thus provide a host system for the production of
recombinant polymeric IgM which will avoid any problems
inherent in the use of cancerous host cells.

Preferably, the non-lymphoid actively secreting cells
are neural cells, such as glial cells or pheochromal
cells.

The advantage of using such a neural cell is that neural
cells have very low amounts of or no histocompatability
antigens. They can therefore be transplanted between
donors and recipients without producing any significant
histocompatibility problems. Thus, a transformed neural
cell could be cultured in vitro and then transplanted
into a recipient so as to enable the recipient to
produce in vivo the recombinant polymeric IgM. This
could have significant medical application.

For instance, a neural cell transformed to enable it to
produce an anti-acetylcholineesterase IgM-type antibody
could be transplanted into the brain of a person
suffering from Alzheimer's disease. The antibody
secreted by the transformed cell may then be able to
alleviate or abolish the symptoms of the disease.

Thus, a transformed neural cell could be used to
circumvent the blood/brain barrier and allow the
secretion in the brain of a useful IgM-type antibody,
for instance directed again a brain tumour antigen or a
viral antigen.

A particular embodiment of the present invention relates
to the use of a glial cell as a host cell for the
production of an assembled polymeric IgM-type
recombinant antibody having a specificity for
acetylcholine esterase. The transformed cell line would
be of use for transplantation into the brain of a person
suffering from Alzheimer's disease.

The recombinant IgM-type molecule produced by the
process of the present invention may be identical to a
natural IgM antibody except that it does not include a
J-chain. Alternatively, it may be a chimeric antibody
having an IgM-type constant region. In a further
alternative it may be a "humanised" antibody having
mouse complementary determining regions grafted onto a
human (or other non-mouse species) IgM molecule
framework regions.

The present invention includes all novel plasmids,
expression vectors, cell lines and products used or
produced in putting the invention into effect.

Processes for producing vectors containing genes
encoding all or part of an Ig chain by recombinant DNA
techniques are now well known in the art. These
techniques are shown, for instance, in the European
patent applications referred to above. General
procedures for producing and manipulating vectors and
transforming cells with the vectors are also well known
and described in a number of textbooks and laboratory
manuals. Also well known in the art are processes for
culturing transformed host cells and recovering the
desired product from the culture. Such processes can
readily be applied to the present invention, given the
teaching of the present application.

The present invention is now described, by way of
example only, with reference to the accompanying
drawings, in which:

Figure 1 shows the structure of plasmid pSV-HSVλ1
wherein the thin line denotes the pSV2neo vector,
sequences of the immunoglobulinλ_1 light chain gene
are stippled, the Drosophila hsp70 promoter region
(phsp70) is hatched, the leader exon derived from the
heavy chain variable region (L$_H$P) is depicted by a
filled box and the open box denotes a 60 nucleotide
stretch of the 5'-untranslated region of tk:

Figure 2 shows the structure of plasmid pSV-HSVμ
2 wherein the various DNA segments are depicted as for
pSV-HSVλ1 except that the vector (thin line) is
pSV2gpt. The DNA encoding the constant region is
derived from a cDNA encoding the Cμ 1, 2, 3 and 4
domains as well as the secreted tailpiece (tp). The
HS-Cμ2 transcription unit uses the SV40
polyadenylation site (SV POLY A) of pSV2gpt;

Figure 3 shows the inducible transcription of
the λ gene in four pools of HeLa cells and in J558L
cells;

Figure 4 shows immunoglobulin secretion from
transfected cells before (-) or after (+) heat-shock and
in the absence or presence of tunicamycin (+ Tm);

Figure 5 shows that IgM secreted from C5 glioma
cells is polymeric;

Figure 6 shows electron micrographs of negatively stained IgM molecules, purified on hapten sorbents, from the supernatant of heat-induced C6 [HSV μ 2/HSV 1] transfectants; and

Figure 7 shows an analysis for the presence of J-chain polypeptide.

In the following description various published papers are referred to by a number in brackets. A list of these papers in numerical order is given at the end of the description.

In Figures 1 and 2, the transcription start sites are indicated by an arrow and the probes used for ribonuclease protection assays (SP6-Vλ and SP6-HSVNP) are also indicated; the SP6-HSVNP   probe lacks the LNP-VNP   intron. Restriction sites are abbreviated: B, BamHI; Bg, BglII; H, HindIII; Ha, HaeII; Hh HaeI; Hh, HhaI; P, PstI; Sa, Sau3AI; Sc, SacI; Sp, SphI; St, StuI; X, XhoI. Note that not all the sites for any one enzyme are shown - only those relevant to plasmid or probe construction are included.

In Figure 3, (A) shows an assay of λ1   mRNA is HeLa transfectants. Ribonuclease protection assays were carried out using the SP6-Vλ probe and cytoplasmic RNA from four pools of HeLa cells stably transfected with pSV-HSVλ1. RNA was prepared from cells before (-) and after (+) heat shock. RNA from the plasmacytoma J558L served as a control and an end labelled HpaII digest of pBR322 provided size markers. The protected fragment (HSλ) is the length of the entire Vλ exon, and (B) shows an assay of HS-Vμ2 RNA in J558L transfectant J[(HSVμ 2]. The ribonuclease protection assay was

carried out on induced (+) and uninduced (-) cells using
a probe for the 5'-end of the HS-V μ 2 transcript as
well as for gpt mRNA.

Plasmids were constructed in which transcription of the
regions encoding Ig heavy and light chains was under the
control of the promoter of the Drosophila hsp70
heat-shock gene.

The Heavy Chain Plasmid

The plasmid for heavy chain expression, pSV-HSV μ 2 is
shown in Figure 1 and includes most of the coding
sequence of the cDNA for an IgM heavy chain with the
hsp70 promoter at the 5' end and the SV40
polyadenylation signal at the 3' end. The transcription
unit also contains a single intron between the regions
encoding most of the leader peptide and the $V_H DJ_H$
region. The transcription unit is linked to a gene
encoding guanidine phosphoribosyl transferase (gpt)
which can be used as a selective marker.

Plasmid pSV-HSV μ 2 includes the HS-V μ 2 transcription
unit cloned in a derivative of pSV2GPT (1) in which the
BamHI site had been converted to a SacI site by use of
linkers. The HS-V μ 2 transcription unit was assembled
in three parts. The promoter/transcription start region
was obtained from plasmid pF1 (2) as an EcoRI-BglII
fragment which contains the Drosophila hsp70 promoter.
The EcoRI-BglII fragment comprised an EcoRI-SalI
fragment extending from the 5' end to immediately 3' of
the TATA in the Drosophila hsp70 promoter. The
EcoRI-SalI fragment is fused to a 60 nucleotide
HaeIII-BglII fragment of the herpes simplex virus (HSV)
tk gene which includes the RNA cap site.

The DNA encoding the leader region of the heavy chain polypeptide (L_{HP}) was obtained from the mouse V_H gene V-47, as a Sau3AI-PstI fragment extending from the transcription start through the leader-V_{NP} intron to the +5 codon of the gene (3). The rest of the heavy chain coding region was derived as a PstI-HaeII fragment of the cDNA clone for the heavy chain of a NP-specific antibody pAB11 (4). The fragment extends from the +5 codon to within the C μ 3'-untranslated region.

After final assembly of pSV-HSVμ2, the HaeII site at the 3' end of C μ is brought adjacent the SacI site in the derivatised pSV2gpt vector. Plasmid pSV-HSV μ 2 is a cDNA derivative of pSV-HSV μ 1 (5).

A probe for mapping HS-V 2 transcripts was obtained by subcloning into pSP65 a fragment derived from an intronless derivative of pSV-HSV 2 extending from the StuI site in V_{NP} to the XhoI site in the hsp70 promoter.

The Light Chain Plasmid

The plasmid for light chain expression, pSV-HSVλ1 is shown in Figure 2. It includes the expressed, genomic λ ₁ gene of the mouse myeloma HOPC 2020 but with the promoter/leader exon replaced by the hsp70 promoter/V_H-leader portion of plasmid pSV-HSVμ2. The HSVλ1 light chain transcription unit is linked to a gene endowing resistance to neomycin (neo) which can be used as a selective marker.

Plasmid pSV-HSV λ 1 is based on a derivative of pSV2neo (6) in which the HindIII site has been destroyed by filling in and a new HindIII site created by linker insertion in the BamHI site. The promoter/transcription

start/leader region is the same as in pSV-HSV μ 2 except
in that (i) the region 5' of the promoter extends only
as far as the SphI site and (ii) the Lₙₚ-Vₙₚ   intron
is truncated at an HhaI site. This HhaI site in the
Lₙₚ   intron is fused to a PstI site in the L λ -v λ
intron of the expressed λ ₁   gene of mouse plasmacytoma
HOPC 2020 (7) which provides the rest of the
transcription unit.

A probe for light chain expression was obtained by
subcloning the BamHI-PstI V λ fragment of the HOPC 2020
light chain gene into plasmid pSP65.

Cell Lines and Transfection

Mouse plasmacytoma cell line J558L (8), its IgM
secreting transfectant JW1/2/2 (9), rat myeloma cell
line YO (10), rat glioma cell line C6 (11), CHO cells
and HeLa cells were grown in DMEM containing 10% FCS.
Pheochromacytoma cells PC12 (12) were grown in RPMI
medium containing 10% heat-inactivated horse serum and
5% FCS.

The phenotype of the C6 glioma cells was confirmed by:
(i) measuring receptors for nerve growth factor (NGF)
(13); fluorescent staining with antibody MC 192 (14);
immunoprecipitation of S100 protein (15); and
immunofluorescent staining for glial fibrillar acidic
protein (16). The PC12 cells were shown to respond to
NGF.

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Transfection of J558L and C6 glioma cells was achieved
using spheroplast fusion, carried out as described by
Neuberger (3). Transfection of PC12 cells was achieved
using electroporation performed by giving four 2kV
pulses to a suspension of 2.5×10^7 cells in 0.5 ml
of supercoiled plasmid DNA (17). Transfection of HeLa
and CHO cells was achieved by calcium-phosphate
coprecipitation.

For selection of neo transfectants, G418 was used at 0.5
mg/ml (PC12), 1 mg/ml (C6) or 2 mg/ml (other cells).
gpt colonies were selected as described by Mulligan and
Berg (1) for non-lymphoid cells and as described by
Neuberger et al. (9) for J558L cells.

Analysis of RNA

Total cytoplasmic RNA was prepared as previously
described by Neuberger (3). For heat induction, RNA was
extracted 4 hours after the cells had been subjected to
2 hour shock at 42.5°C. Ribonuclease protection
assays were performed as described by Melton et al. (18).

Analysis of Protein

For biosynthetic labelling of immunoglobulin, cells (5 x
10^6 in 1 ml) were heat-shocked at 42.5°C for 2
hours, washed and resuspended in medium containing 5%
dialysed FCS, one-tenth the normal concentration of
methionine and 50 μ Ci/ml L-^{35}S]methionine (1200
ci/mmol; Amersham International). After a 14 - 16 h
incubation at 37°C, cells were collected by
centrifugation, washed and lysed in 0.2 ml PBS/0.5%
Nonidet-P40/1mM phenylmethylsulphonyl fluoride (PMSF).

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For analysis of J chain, the labelling period was
shorter and cells were lysed in 0.1M-Tris.HCl (pH 8.0)
/0.1M-KCl/5 mM-MgCl₂ 0.5%   NP40/1mM-PMSF.   When
required, tunicamycin (8 μ g/ml) was included during the
heat-shock and labelling periods.

Immunoprecipitations from culture supernatants or
cytoplasmic extracts were carried out by incubation and
purified rabbit anti-mouse μ antiserum or rat monoclonal
antibody SM1/45, goat anti-mouse λ monoclonal
anti-idiotypic antibodies Ac38 and Ac146 (19) or rabbit
anti-mouse J chain antiserum, followed by precipitation
with protein A-Sepharose, (Pharmacia). After washing
with 50 mM-Tris.HCl (pH 7.6)/ 1M-NaCl/0.25% NP40,
samples were dissolved in reducing SDS sample buffer and
analysed by electrophoresis through SDS/polyacrylamide
gels (23). Unreduced samples were analysed on 4%
acrylamide/0.7% N,N'-diallyltartardiamide gels (24).
Purification on hapten sorbents was carried out using
NIP-caproate linked to Sepharose as previously described
(9).

For analysis in the electron microscope, antibody that
had been freshly affinity purified on hapten columns was
concentrated to 1 mg/ml, layered onto carbon films and
viewed by negative contrast following staining with
uranyl acetate.

Cell lines were transfected with either plasmid pSV-HSV
μ 2 alone (J558L) or with plasmids pSV-HSVμ2 and
pSV-HSV λ 1 together (other cells). Association of the
polypeptides encoded by the two plasmids should yield an
IgM λ ₁   antibody with specificity for the hapten
4-hydroxy-3-nitrophenacetyl (NP). Stable transfectants

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were screened for the expression of Ig gene mRNA and
protein. The results are shown in Table 1, where they
are compared with the result from the JW1/2/2 line which
secretes IgM constitutively.

Table 1

Antibody yields from transfected cells.

Cell Line	Cell-type	Concentration of IGM μ g.ml).
J558L3	plasmacytoma	0.5
C6	glioma	0.6
PC12	Pheochromocytoma	0.01
CHO	Fibroblast	0.01
HeLa	Epithelial	0.005
JW1/2/2	Plasmacytoma	3.5

3×10^6 cells/ml (initial concentration) were
incubated at 37°C   for 3 days with 1 hour heat shocks
at 42.5°C   every 24 hours. The concentration of IgM
was determined by radioimmunoassay on antigen-coated
plates using purified JW1/2/2 IgM for calibration.

Four different pools of HeLa cells were transfected with
pSV-HSVλ 1 and three showed inducible λ gene

transcription. The fourth pool transcribed the gene
constitutively. These results are shown in Figure 3A.
Ribonuclease protection assays (18) were carried out
using the SP6-V λ probe and cytoplasmic RNA from the
pools of HeLa cells stably transfected with pSV-HSV λ 1.
RNA was prepared from cells before (-) and after (+)
heat shock. RNA from J558L served as a control and an
end-labelled HpaII digest of pBR322 provided size
markers. The protected fragment (HS λ) is the length of
the entire V λ exon.

Similarly, pSV-HSV μ 2 transfectants of J558L cells
showed inducible transcription of the inducible heavy
chain gene. The ribonuclease protection assay was
carried out on induced (+) and non-induced (-) cells
using the probe for the 5' end of the HS-V μ 2 as well
as a probe for gpt mRNA. The size of the fragment
obtained in the RNase protection assay indicates that
the transcripts were initiated by the Hsp70 promoter.
The result is shown in Figure 3B.

It was confirmed by Northern blotting that the HSV μ 2
and HSV λ 1   transcripts were of the expected size.

Secretion of glycosylated IgM by non-lymphoid transfectants

Analysis of cells transfected with plasmid pSV-HSV λ 1
revealed that most of these transfectants secreted λ 1
light chains into the culture supernatant. Clones of
the transfected rat pheochromocytoma cell line PC12 and
of the rat glioma cell line C6 were analysed for
polypeptide synthesis. Whilst there was clonal

variation in the amount of λ expressed (some neo[+] clones
being negative for λ_1 expression), it was clear from
immunoprecipitation experiments that heat induction of
many of the C6 and PC12 transfectants resulted in the
secretion of light chains. The results are shown in
Figure 4A which shows light chain secretion by J558L and
C6 and by pSV-HSVλ1 transfectants of C6 and PC12.
Samples were immunoprecipitated from culture
supernatants using anti-antiserum. Interestingly, the
amount secreted by these transfectants is comparable to
the amount of constitutive light chain secretion by the
mouse plasmacytoma J558L, despite the fact that J558L
contains appreciably more λ_1 mRNA.

In order to test whether the pheochromocytoma and glioma
lines were able to assemble complete immunoglobulin,
biosynthetically labelled proteins of PC12 and C6 cells
that had been transfected with both pSV-HSV μ 2 and
pSV-HSV λ 1 were analysed. The results are shown in
Figure 4B which shows intracellular immunoglobulin
production in PC12 and C6 expression plasmids. After
biosynthetic labelling, cytoplasmic samples were
immunoprecipitated with anti- μ antiserum. These
results show that heat shocking of both cell lines
results in induction of the synthesis of μ
polypeptide. The μ heavy chain is associated with
$_1$ λ light chain and is glycosylated as judged by the
effect of the glycosylation inhibitor tunicamycin.

The C6 glioma transfectants were able to secrete the
associated μ and λ polypeptides. This is shown by
Figure 4C which shows the secretion of IgM by C6[HSV
1/HSV μ 2] transfectant and by constitutive

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IgM-secreting J558L transfectant, JW1/2/2. After
biosynthetic labelling, samples from the supernatant
were precipitated with anti- μ antiserum. The high
molecular weight band in this gel that is indicated by
an arrow is also precipitated from untransfected C6
cells by anti- μ antibodies and reflects serological
cross-reaction. The other arrow indicates the dye-front.
The antibody secreted by these glioma transfectants was
similar to that secreted by the plasmacytoma
transfectant JW1/2/2 in that both antibodies bound the
hapten NP. This is shown in Figure 4D which is a
comparison of IgM secretion by JW1/2/2 (constitutive
plasmacytoma expression), C6[HSV λ 1/HSV μ 2]
(heat-inducible glioma transfectant) and J[HSV μ 2]
(which is a pSV-HSV μ 2 transfectant of J558L and is
therefore a heat-inducible IgM secreter). All samples
were prepared following heat shock and analysed in
parallel except that only half the JW1/2/2 sample was
loaded on the gel and an incubation of J[HSV μ 2] cells
in the absence of heat-shock was performed for the right
hand lane. IgM from culture supernatants was purified
by adsorption onto NIPcap-Sepharose and eluted with 1
mM-NIPcapOH.

Both antibodies were recognized by anti-idiotypic
antibodies. Figure 4E shows immunoprecipitation of
glioma IgM with anti-idiotypic antibodies.
Immunoglobulin in the supernatant of biosynthetically
labelled C6[HSV μ 2/HSV μ 1] cells prior to (-) or
following (+) heat shock was precipitated using
monoclonal anti-idiotypic antibody Ac38 or Ac146 (19).
Furthermore, the extent of glycosylation of the μ
polypeptide secreted by the two cell-types appears
similar as judged by mobility in SDS/polyacrylamide gels.

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To compare the efficiency of antibody secretion by a
glioma transfectant to that by a plasmacytoma, we used
the C6 double transfectant C6[HSV μ 2/HSVλ1] and the
J558L transfectant J[HSV μ 2], which expresses an
inducible μ gene from the transfected pSV-HSV   2
plasmid and constitutively transcribes its endogenous
ι   gene. In both transfectants, heat shocking leads to
induction of μ synthesis in at least 80% of the cells as
judged by cytoplasmic immunofluorescence. Following
periodic heat pulses, the concentration of
hapten-specific IgM in the culture supernatants was
estimated by radioimmunoassay.

The results (Table 1) confirm that the level of
inducible secretion is similar in the glioma and
plasmacytoma transfectants - a conclusion which is in
keeping with the results of biosynthetic labelling
(Figure 4D). However, it is worth noting that the
antibody yields obtained from the HeLa and CHO cell
transfectants were considerably lower (Table 1).

IgM secreted by glioma transfectants is polymeric and
does not contain J chain.

As the conventional wisdom is that J chain is necessary
for IgM assembly and secretion and as J chain expression
appears to be restricted to mature cells of the B
lymphocyte lineage, it was important to ascertain
whether the IgM secreted by the glioma transfectants
contained immunoglobulin J chain.

To determine whether there was any intracellular J chain
in C6 cells, immunoprecipitation was carried with an
anti-J chain antiserum using cytoplasmic extracts of

biosynthetically labelled cells. The results are shown
in Figures 7A and 7B. A rabbit antiserum against J
chain was used to precipitate J chain from
biosynthetically labelled cell extracts that had been
made in the presence or absence of tunicamycin (Tm) and
that had been pre-cleared with anti-light and anti-heavy
chain antisera and protein A-Sepharose. Nethertheless,
it will be seen that precipitation with the anti-J chain
antiserum does bring down some residual immunoglobulin
polypeptides. Figure 7A shows the results obtained with
Rat YO myeloma cells and cells of the C6 [HSV μ 2/HSV
1] transfectant that had been heat-induced 5 hours
previously were incubated in labelling medium for 5
hours. Figure 7B shows the results obtained with
JW1/2/2 cells and cells of the J[HSV μ 2] and C6[HSV μ
2/HSVλ 1] transfectants that had been heat-induced 7
hours previously and were incubated in labelling medium
for 1 hour.

These results show that J chain can be detected in
extracts of the plasmacytoma transfectants but not in
the C6 glioma sample. Various different labelling times
were used as it has been reported (21) that the
half-life of J chain differs considerably in different
cell-lines. The detection of J chain polypeptide in the
YO rat myeloma demonstrates that the anti-mouse J chain
antiserum that was used will indeed precipitate rat J
chain antiserum that was used will indeed precipitate
rat J chain - a point that needed confirming as C6 is a
glioma of rat origin. Not only was no J chain
polypeptide detected in C6 cells, but it also appeared
that these cells did not contain J chain mRNA as judged

by primer extension assays. Furthermore, analysis of
IgM secreted by the plasmacytoma and C6 transfectants in
alkaline/urea polyacrylamide gels after reduction and
alkylation revealed J chain to be present only in the
plasmacytoma sample.

The sizes of the IgM secreted by the plasmacytoma and
glioma transfectants were compared using both sucrose
gradient centrifugation (Figure 5A) and electrophoresis
through non-reducing SDS/polyacrylamide gels (Figure 5B)

In the Analysis by sucrose gradient centrifugation,
supernatants (1 ml) from biosynthetically labelled
C6[HSV μ 2/HSV λ 1] (+------+), plasmacytoma
IgM-secreting transfectant JW1/2/2 (o———o) or
IgG1-secreting hybridoma P8.86.9 were loaded onto a
gradient (15ml) of 10% sucrose in PBS and subjected to
centrifugation in a Beckman SW40 rotor at 4°C   for 18
hours. Samples were collected, the refractive indices
determined and anti-NP antibody measured by
radioimmunoassay. The supernatants from the C6 and
J558L transfectants were analysed in parallel.

In the analysis by polyacrylamide gel electrophoresis,
anti-NP antibody in the supernatants of biosynthetically
labelled JW1/2/2 or of heat-induced C6[HSV μ 2/HSV λ1]
was purified on hapten sorbents and analysed either
after reduction (left panel) on a 7.5%
SDS/polyacrylamide gel or unreduced (right panel) on a
4%polyacrylamide gel made using
N,N'-diallyltartardiamide to cross-link. The positions
of the origin, IgM, IgE, IgG, and μ markers are
indicated.

In both analyses, the IgM secreted by the glioma
transfectant behaved exactly like the IgM secreted by
the plasmacytoma and revealed itself to be a high
molecular weight, covalently-associated aggregate. the
resolution of the centrifugation and gel electrophoresis
experiments was not, however, sufficient to demonstrate
that the polymeric IgM secreted by the glioma
transfectants was of exactly the same size as that
produced by the plasmacytoma cells. An analysis of
negatively stained protein samples was carried out using
electron microscopy, the results of which are shown in
Figure 6. Different fields are shown in which both
hexamers (H) and pentamers (P) are visible. Comparison
of the IgM samples prepared from plasmacytoma and glioma
transfectants shows that whereas the IgM secreted by the
plasmacytoma is almost exclusively pentameric, the
sample prepared from the glioma transfectants is found
to contains both cyclic hexamers and cyclic pentamers in
approximately equal amounts. The sample prepared from
C6 cells also contains some molecules which, at first
sight, appear to be incompletely assembled structures.
However, the same structures are seen in the
plasmacytoma IgM sample and are most probably hexamers
or pentamers that are not lying flat on the grid (22).
Analysis of IgM secreted from the heat-inducible
plasmacytoma transfectant J[HSV μ 2] revealed it to be
exclusively pentameric, indicating that the presence of
hexamers in the glioma sample is unlikely to be an
artefact of heat shocking.

Finally, it worth noting that pulse-chase experiments
performed using biosynthetic labelling revealed that the
polymerization of the IgM molecules occurred prior to
their secretion from the glioma or plasmacytoma cell.

Discussion

Several different non-lymphoid cell-lines were
transfected with immunoglobulin heavy and light chain
genes whose expression was under control of a heat-shock
promoter. Transfectants were obtained that showed
inducible expression, demonstrating that non-lymphoid
cells are capable of assembling, processing and
secreting immunoglobulin. There was considerable
variation in the yield of immunoglobulin obtained using
different cell-types as host. Whereas HeLa and CHO
cells gave relatively poor yields, the amounts of
antibody secreted by glioma and pheochromacytoma
transfectants were comparable to those obtained using a
plasmacytoma host. The greater efficacy of C6 glioma or
PC12 as opposed to CHO or HeLa transfectants in antibody
secretion does not appear to be due to a difference in
the induction of immunoglobulin mRNA synthesis but
reflect the fact that the glioma and pheochromocytoma
are actively secreting cell types and may therefore be
better equipped for the production of secreted
immunoglobulin.

The IgM secreted by the C6 transfectants is polymeric,
notwithstanding the absence of immunoglobulin J chain.
This was somewhat unexpected as it has been proposed

that J chain is required for the assembly and secretion
of IgM. IgM secreted by the glioma transfectants is
part pentameric, part hexameric and it is conceivable
that the presence of hexamers is a direct consequence of
the absence of J chain. Therefore it is clear that J
chain is not required for the production of polymeric
IgM in good yield from the glioma transfectants.

Both PC12 and C6 cells are derived from cell types
present in the nervous system. The fact that
transfectants of both cell types are able to synthesize
and secrete immunoglobulin means that it should be
possible to engineer the production of specific
antibodies in the central nervous system of transgenic
organisms in order to perturb or modulate the activity
of selected neuronal pathways or to effect medication.

It will of course be appreciated that the present
invention has been described above purely by way of
illustration and that modifications and alterations of
detail can be made by the skilled man without departing
from the scope of the invention.

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CLAIMS

1.   A process for the production of an assembled
polymeric recombinant antibody having its constant
regions derived from an IgM, (or IgM-type antibody),
characterised in that the process is carried out in the
absence of J-chain or any active gene coding therefor.

2.   The process of claim 1, wherein a host cell
lacking a J-chain encoding gene or having an inactive
J-chain encoding gene is transformed with a vector
containing a gene encoding an IgM-type heavy chain and a
gene encoding a complementary light chain.

3.   The process of claim 1, wherein a host cell
lacking a J-chain encoding gene or having an inactive
J-chain encoding gene is transformed with a vector
containing a gene encoding an IgM-type heavy chain and a
vector encoding a complementary light chain.

4.   The process of any one of claims 1 to 3, wherein
the host cell is a non-lymphoid actively secreting cell.

5.   The process of claim 4, wherein the actively
secreting cell is an exocrine cell or a neural cell.

6.   The process of claim 5, wherein the actively
secreting cell is a glial cell.

7.   The process of any one of claims 1 to 6, wherein
the IgM-type antibody has an anti-acetylcholine esterase
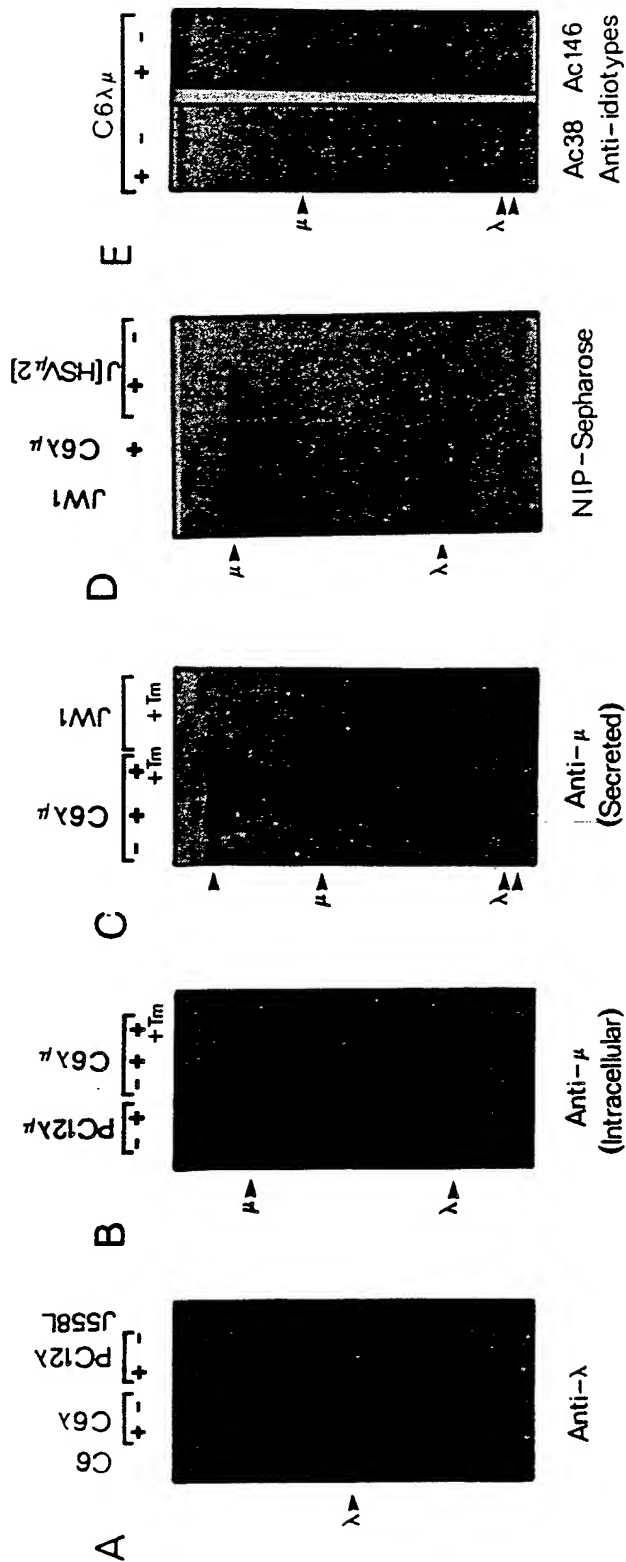specificity.

-27-

8.   A non-lymphoid actively secreting cell which has been transformed such that it can express an IgM-type antibody.

9.   An IgM-type antibody substantially free of J-chain.

10.   The IgM-type antibody of claim 9 which is a natural, chimeric or humanised antibody.

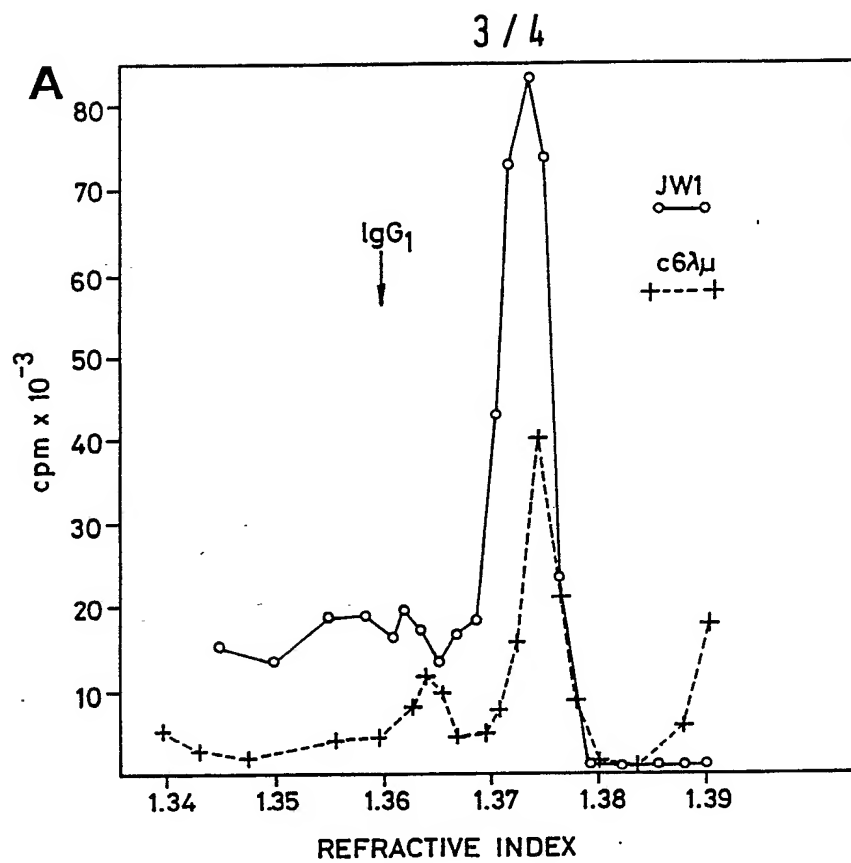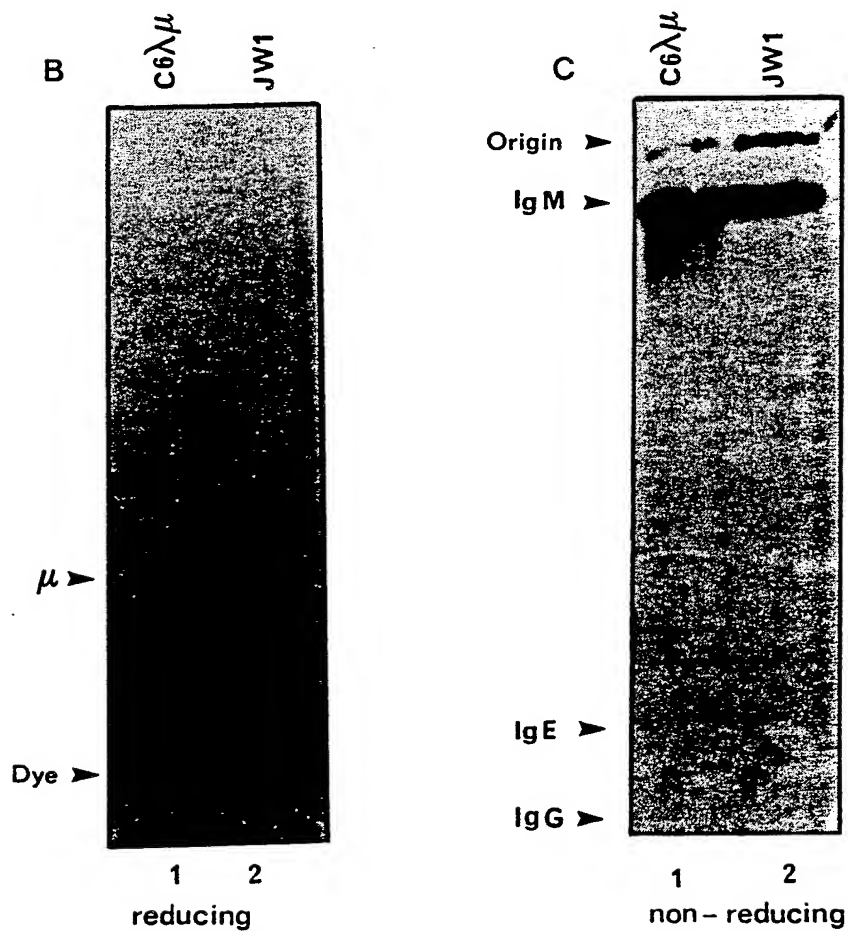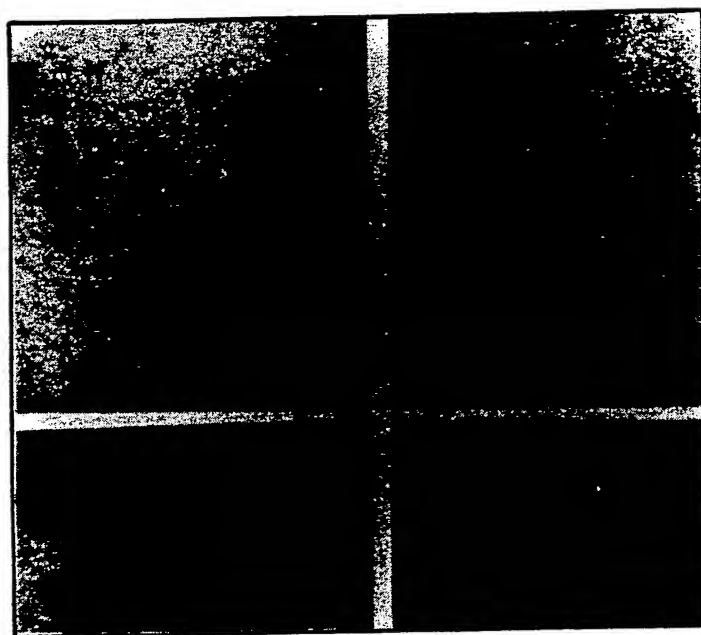pSV-HSV$_\lambda$1　　　　　　　　　　　　　1 / 4

FIG.1

$\llcorner$L$_{NP}$ V$_\lambda$　　　　　　C$_\lambda$　　　　　　　　　　amp　　neo

phsp 70

H Sp　Hh/P　　　　B　　　　　　　R

Bg/Sa

　　　　　　　　　　　　　　　　　　　　500 bp

$\longleftarrow$ SP6-V$_\lambda$

pSV-HSV$_\mu$2

　　　　　　　　　　　L$_{NP}$ V$_{NP}$　　C$_\mu$

FIG.2

　　　　　　　　　　　　　　　1　2　3　4 tp

phsp 70　　　　　　　　　　　　　　　　SV POLY A　gpt　amp

R　　　Sp X Bg/Sa St　　　　　　　Ha/Sc

$\longleftarrow$ SP6-HSV$_{NP}$

FIG.3

A

probe
markers
J558L
HeLa
HeLa [HSV$_\lambda$1]
− + − + − + − +

B

markers
probes
+] J[HSV$_\mu$2]
−

HS$_\mu$ ►

gpt ►

HS$_\lambda$ ►

FIG.4

FIG.5

FIG.6

FIG.7

A

B

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁴: C 12 P 21/00; C 12 N 5/00; A 61 K 39/395; C 12 N 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC⁴	C 12 N; C 12 P

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	Nature, volume 311, 6 September 1984, P. Brandtzaeg et al.: "Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins", pages 71-73 see page 71, column 2, paragraph 3; table 1 last line --	1,9,10
A	Gene, volume 51, no. 1, April 1987, Elsevier Science Publishers B.V. (Biomedical Division), U.H. Weidle et al.: "Reconstitution of functionally active antibody directed against creatine kinase from separately expressed heavy and light chains in non-lymphoid cells", pages 21-29 see the whole article --	1-4,8-10
A	Nature, volume 314, 4 April 1985, ./.	1-3,9,10

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd December 1988	2 9 DEC 1988

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT   (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	C.R. Wood et al.: "The synthesis and in vivo assembly of functional antibodies in yeast", pages 446-449 see page 448, paragraph 1	
P,X	The EMBO Journal, volume 6, no. 9, September 1987, IRL Press Ltd, (Oxford, GB), A. Cattaneo et al.: "Polymeric immunoglobulin M is secreted by transfectants of non-lymphoid cells in the absence of immunoglobulin J chain", pages 2753-2758 see the whole article	1-6,8-10